

## CLAIMS

1. A composition for detecting  $\beta$ -1,3-glucan exhibiting phenoloxidase activity by  $\beta$ -1,3-glucan in the presence of calcium ions, prepared by the method  
5 comprising:

collecting a sample comprising a mixture of plasma and hemocyte lysate from an insect;

treating said sample with a solvent or buffer solution containing a sufficient amount of a chelating agent to chelate calcium ions existing in said  
10 sample and during a separation process to obtain fractions therefrom; and

selecting fractions exhibiting phenoloxidase activity by  $\beta$ -1,3-glucan in the presence of calcium ions from the obtained fractions.

2. A composition for detecting  $\beta$ -1,3-glucan the minimum down to 20 pg/ml in the  
15 presence of calcium ions.

3. A method of preparing a phenoloxidase composition activated by  $\beta$ -1,3-glucan in the presence of calcium ions, comprising:

collecting a sample comprising a mixture of plasma and hemocyte lysate  
20 from an insect;

treating said sample with a solvent or buffer solution containing a sufficient amount of a chelating agent to chelate calcium ions existing in said sample and during a separation process to obtain fractions therefrom; and

selecting fractions exhibiting phenoloxidase activity by  $\beta$ -1,3-glucan in the

presence of calcium ions from the obtained fractions.

4. The method according to Claim 3 wherein said insect belongs to Coleoptera.

5 5. The method according to Claim 4 wherein said Coleoptera belongs to Tenebrionidae or Scarabaeidae.

6. The method according to Claim 3 wherein said fractions are obtained by column chromatography.

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7. The method according to Claim 6 wherein the column used for said column chromatography is packed with a resin comprising dextran or vinyl.

8. The method according to Claim 3, wherein whole or partially purified  
15 hemocyte lysate is further added to the fractions exhibiting the phenoloxidase activity by  $\beta$ -1,3-glucan in the presence of calcium ions.

9. A method of preparing a phenoloxidase composition activated by  $\beta$ -1,3-glucan in the presence of calcium ions, comprising:

20       treating insect plasma with a solvent or buffer solution containing a sufficient amount of a chelating agent to chelate calcium ions existing in said plasma and during a separation process to obtain fractions therefrom;

      adding hemocyte lysate or partially purified hemocyte lysate to said fractions; and

selecting fractions exhibiting phenoloxidase activity by  $\beta$ -1,3-glucan in the presence of calcium ions.

10. The method according to Claim 9 wherein said insect belongs to Coleoptera .

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11. The method according to Claim 10 wherein said Coleoptera belongs to Tenebrionidae or Scarabaeidae.

12. The method according to Claim 9 wherein the said fractions are obtained by column chromatography.

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13. The method according to Claim 12 wherein the column used for said column chromatography is packed with a resin comprising dextran or vinyl.

14. The method according to Claim 9, wherein whole or partially purified hemocyte lysate is further added to the fractions exhibiting the phenoloxidase activity by  $\beta$ -1,3-glucan in the presence of calcium ions.

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15. A method of detecting  $\beta$ -1,3-glucan comprising the steps of:

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collecting a sample from a specimen;

adding a composition of Claim 1 and calcium ions to said sample; and

measuring the phenoloxidase activity in the sample.

16. A diagnostic kit for detecting  $\beta$ -1,3-glucan which contains the composition detecting  $\beta$ -1,3-glucan the minimum down to 20 pg/ml in the presence of calcium ions.

5 17. A diagnostic kit for detecting  $\beta$ -1,3-glucan exhibiting phenoloxidase activity by  $\beta$ -1,3-glucan in the presence of calcium ions, prepared by the method comprising:

collecting a sample comprising a mixture of plasma and hemocyte lysate from an insect;

10 treating said sample with a solvent or buffer solution containing a sufficient amount of a chelating agent to chelate calcium ions existing in said sample and during a separation process to obtain fractions therefrom; and

selecting fractions exhibiting phenoloxidase activity by  $\beta$ -1, 3-glucan in the presence of calcium ions from the obtained fractions.

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18. The diagnostic kit according to Claim 17 wherein said insect belongs to Coleoptera.

19. A diagnostic kit for detecting  $\beta$ -1,3-glucan exhibiting phenoloxidase activity  
20 by  $\beta$ -1,3-glucan in the presence of calcium ions, prepared by the method comprising:

treating insect plasma with a solvent or buffer solution containing a sufficient amount of a chelating agent to chelate calcium ions existing in the plasma and during a separation process to obtain fractions;

adding hemocyte lysate or partially purified hemocyte lysate to the above obtained fractions; and

selecting fractions exhibiting phenoloxidase activity by  $\beta$ -1, 3-glucan in the presence of calcium ions.

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20 . The diagnostic kit according to Claim 19 wherein the insect belongs to Coleoptera .

21 . A composition for detecting  $\beta$ -1,3-glucan exhibiting phenoloxidase activity by  
10  $\beta$ -1,3-glucan in the presence of calcium ions, prepared by the method comprising:

treating insect plasma with a solvent or buffer solution containing a sufficient amount of a chelating agent to chelate calcium ions existing in the plasma and during a separation process to obtain fractions therefrom;

15 adding hemocyte lysate or partially purified hemocyte lysate to said fractions; and

selecting fractions exhibiting phenoloxidase activity by  $\beta$ -1,3-glucan in the presence of calcium ions.

20 22 . A method of detecting  $\beta$ -1,3-glucan comprising the steps of:

collecting a sample from a specimen;

adding a composition of Claim 21 and calcium ions to said sample; and

measuring the phenoloxidase activity in the sample.

23 . The composition according to Claim 2 wherein said composition is characterized in measuring the phenoloxidase activity.

24 . The diagnostic kit according to Claim 16 wherein said diagnostic kit is  
5 characterized in measuring the phenoloxidase activity.